Genetic Variants in Serotonin and Corticosteroid Systems Modulate Neuroendocrine and Cardiovascular Responses to Intense Stress

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Research report

Genetic variants in serotonin and corticosteroid systems modulate neuroendocrine and cardiovascular responses to intense stress☆

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HIGHLIGHTS

- This study revealed a remarkable synergistic effect of common polymorphisms on acute stress response in healthy men.
- 5HTTLPR SS carriers revealed higher overall cortisol concentrations than L carriers in response to intense, realistic stress.
- 5HTTLPR S carriers showed higher overall DBP values than non-carriers (LL), Bcl1 GG were higher than C carriers, and −2C/G G carriers exceeded non-carriers (CC).
- A "high" genotype group revealed substantially higher overall cortisol concentrations than a "low" genotype group, as was the case for DBP.

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ABSTRACT

Common variants in serotonin and corticosteroid receptor genes influence human stress in laboratory settings. Little is known of their combined effects, especially in high stress environments. This study evaluated distinct and combined effects of polymorphisms in the serotonin transporter (5HTTLPRL/S), glucocorticoid receptor (Bcl1C/G), and mineralocorticoid (−2C/G) receptor genes on adrenocortical and cardiovascular responses to intense, realistic stress. One hundred and forty four healthy, active-duty military men were studied before, during, and 24 h after a stressful 12-day survival course. Dependent variables were cortisol, heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP). 5HTTLPR SS carriers revealed higher overall cortisol concentrations than L carriers ($p = .022$). 5HTTLPR L carriers demonstrated higher stress-induced HR than non-carriers (SS) yet rebounded to a lower recovery value ($p = .026$), while Bcl1 G carriers showed higher mean stress-induced HR than non-carriers (CC) ($p = .047$). For DBP, 5HTTLPR S carriers showed higher overall values than non-carriers (LL) ($p = .043$), Bcl1 GG were higher than C carriers ($p = .039$), and −2C/G G carriers exceeded non-carriers (CC) ($p = .028$). A "high" composite genotype group revealed substantially higher overall cortisol concentrations than a "low" composite genotype group ($p < .001$), as was the case for DBP ($p = .037$). This study revealed a synergistic effect of common polymorphisms on the acute stress response in healthy men. Pending additional study, these findings may have implications for drug discovery, gene therapy, and stress inoculation strategies.

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1. Introduction

Psychological and physiological stressors ignite a common cascade of autonomic, neuroendocrine, and cardiovascular events, collectively termed "fight or flight" [1]. A well-known mediator of this response is the hypothalamic–pituitary–adrenal axis. Although transient stress responses can be adaptive, repetitive responses across the life span may precipitate psychosomatic and metabolic disease (1). It is therefore crucial to understand individual differences in acute stress reactivity. Key individual characteristics modulating the hypothalamic–pituitary–adrenal axis are found in the transporters, receptors, and related cellular proteins that mediate or modulate corticosteroid action [2].

One such protein, the 5-hydroxytryptamine (i.e., serotonin) transporter (5HTT), facilitates presynaptic serotonin uptake. Human 5HTT is influenced by a single gene (SLC6A4; location 17q11.1-q12) [3] within which transcriptional activity is governed by a 5′ regulatory region (i.e., serotonin transporter gene-linked polymorphic region, or 5HTTLPR). The majority of its alleles contain either a 44-base pair (bp) allelic insertion (long) or deletion (short) [4]. The 5HTTLPR polymorphism influences both activation and feedback control of the hypothalamic–pituitary–adrenal axis [5], and it has been shown to modulate the effect of stressful life events on depression and suicidality [6]. Both experimental [5,7,8] and meta-analytic evidence [9] link the S allele to higher and/or more prolonged acute stress responses. There is far less consensus, however, regarding the association between 5HTTLPR and cardiovascular stress reactivity [10–12].

Another influential protein is the glucocorticoid receptor (GR; encoded by NR3C1, location 5q31-32) [13], which binds glucocorticoids and triggers termination of the stress response [14]. A common GR variant is the Bcl1C/G restriction fragment length polymorphism. This polymorphism affects cortisol feedback regulation [15] and is implicated in hypertension [16], abdominal obesity [17], bone resorption [18], and depression [19]. Wust et al. [20] found diminished cortisol responsivity in healthy male GG subjects in response to psychosocial stress. Kumsta et al. [21] showed diminished reactivity in GG men yet *higher* cortisol responses in GG women, although a subsequent report [22] showed diminished reactivity among GG in both sexes. None of these patterns were replicated in a large study of adolescents that employed rigorous post hoc corrections for multiple comparisons [23]. In terms of metabolic and cardiovascular endpoints, Srivastava et al. [15] showed that G carriers had higher waist–hip ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), and insulin and glucose values than non-carriers in obese subjects, along with higher DBP and glucose in non-obese subjects. The role of GR in responses to highly realistic stress is not known.

A third prominent protein, the mineralocorticoid receptor (MR; encoded by NR3C2, location 4q31.1-31.2) [24,25] is activated by both mineralocorticoids and glucocorticoids, mediates electrolyte balance and blood pressure regulation [26], and governs the threshold for *onset* of the stress response. Dysfunctional MR activation is implicated in cardiac disease [27]. A well-characterized MR polymorphism, −2C/G is located in the 5′ region of NR3C2. The C allele has been linked to lower basal cortisol levels [28,29], while GG is associated with renin–angiotensin system activation and increased SBP [26]. Little is known of the role of this polymorphism in adrenocortical or cardiovascular stress *reactivity*, particularly with respect to highly intense stress exposure.

The purpose of this study was to prospectively evaluate unique and combined effects of candidate polymorphisms in the serotonin transporter (5HTTLPRL/S), glucocorticoid receptor (Bcl1C/G), and mineralocorticoid receptor (−2C/G) genes on adrenocortical and cardiovascular stress responses in healthy military men. It was hypothesized that 5HTTLPR would modulate cortisol responses (S>L), Bcl1C/G would affect cortisol (C>G) and blood pressure responses (G>C), while MR −2C/G would influence baseline and recovery cortisol and blood pressure (both G>C). Additive effects of these three polymorphisms were anticipated.

2. Materials and methods

2.1. Military survival training

Survival, Evasion, Resistance, and Escape (SERE) training is described in earlier reports, [30,31]. United States military members who are deemed "high risk of capture" are required to attend this course, which includes a period of mock captivity. After an initial phase of classroom-based didactic training (5 days), students are taken to a field site where they are trained in survival, evasion, resistance, and escape techniques (7 days). Training tasks include evasion from a simulated enemy and, upon eventual "capture," students must practice resistance to various forms of simulated exploitation in stressful mock-captivity training challenges. The entire course lasts 12 days, including 1 debrief day after the conclusion of mock captivity. Accruing evidence confirms it is a categorically stressful context, quantified by severe disruption of physiological and self-report indices [30,31].

2.2. Inclusion, exclusion, and compliance criteria

Subjects met inclusion criteria if they were active duty military members enrolled in SERE training at the Center for Security Forces, SERE Learning Site West (San Diego, CA), as part of their military duties and were deemed healthy, as indicated by a medical records review conducted by the SERE medical officer. Additional exclusion criteria imposed for this study included smoking; caffeine dependence; any use of anabolic (e.g., dehydroepiandrosterone, growth hormone) or ergogenic substance, drug, or supplement (e.g., creatine monohydrate) within the past 3 months; current antihypertensive medication use (e.g., beta-blockers); and current diagnosis of type 1 diabetes or type 2 diabetes and treated with prescribed medication.

Compliance requirements were imposed during baseline and recovery assessments. Specifically, subjects were asked to refrain from alcohol ingestion within 12 h of assessments, major meals within 1 h of assessments, and caffeine ingestion within 30 min of assessments. Compliance during mock captivity was implicitly controlled by the training context.

2.3. Participants

One hundred and fifty-six military men were originally enrolled in a larger study evaluating stress and health in survival trainees. Twelve were excluded from the current study because they were delayed at a collection point directly after the mock-captivity event by >3 min (autonomically induced cardiovascular stress responses are known to dissipate substantially within 2–3 min of acute stress exposure [32]). This yielded a sample size of 144. This protocol was approved by the Naval Health Research Center Institutional Review Board.

2.4. Protocol

Participants completed baseline salivary and cardiovascular assessments on the first day of the academic phase of SERE training (Time 1 [T1]; pre-stress). Subsequently, all subjects experienced a rigorous evasion exercise, and then participated in a highly realistic mock-captivity scenario. Assessments were performed again directly after a stressful mock-captivity event (Time 2 [T2]; mock-captivity stress). Finally, approximately 24 h after release from

mock captivity (marking completion of field training), assessments were completed a third time (Time 3 [T3]; recovery).

2.4.1. Cardiovascular and salivary assessments

The Finger Pulse Oximeter (MedSource International, Mound, MN) was used to assess heart rate (HR), and blood pressure (BP) was assessed via acoustic sphygmomanometer [33,34]. All BP recordings were estimated to the nearest 2 mm Hg. Two members of the research team performed the BP measurements. Inter-rater reliability established in a convenience sample of 10 subjects (70% male) was very high (SBP, $r = 0.98$; DBP, $r = 0.96$). Salivary sampling occurred after BP measures. At T2, this was approximately 8–20 min after termination of the acute mock-captivity challenge, depending on individual salivary flow rates. At each time point, subjects provided a salivary sample using the passive drool technique [35]. Mean collection times of day for T1, T2, and T3 were 1207, 1440, and 1208, respectively.

2.4.2. Biochemical analysis

All samples were assayed for salivary cortisol in duplicate using a highly sensitive enzyme immunoassay (Salimetrics, LLC, State College, PA). The test uses 25 μL of saliva per determination, has a lower limit of sensitivity of 0.003 μg/dL, standard curve range from 0.012 μg/dL to 3.0 μg/dL, an average intra-assay coefficient of variation of 3.5%, and an average inter-assay coefficient of variation of 5.1%. Method accuracy determined by spike recovery averaged 100.8%, and linearity determined by serial dilution averaged 91.7%. Serum–saliva correlations from a normative database (Salimetrics) show the expected strong linear relationship ($r = 0.91$, $p < .0001$; $n = 47$).

2.4.3. Determination of genotypes

A modified Puregene (Gentra) extraction method was used to isolate the DNA from the saliva samples. For Bcl1 and −2G/C single nucleotide polymorphisms, a TaqMan genotyping assay (7500 Real-Time PCR System, Applied Biosystems, Inc., Foster City, CA) was employed to amplify and evaluate the two alleles at their respective target locations. To conduct variable number tandem repeat (VNTR) analyses for 5HTTLPR, DNA templates were amplified for the region of interest using polymerase chain reaction (PCR) and VNTR-specific fluorescent-labeled primers. The amplified DNA products were then analyzed by capillary electrophoresis to detect the number of repeats present, which was then used to categorize the VNTR genotype. A 22-bp repeat (44-bp insertion/deletion) located in the promoter region was investigated following the method used by Wendland et al. [36].

2.4.4. Statistical analysis

Data were analyzed using SPSS software, version 19.0 (SPSS, Inc., Chicago, IL). Distribution characteristics for all continuous variables were examined to determine if assumptions of normality were met, following conservative predefined limits (e.g., skewness between −1 and 1 [37], kurtosis between −3 and 3). Variables exceeding any of these limits were transformed prior to performing the relevant statistical test. All data transformations reduced skewness and kurtosis to acceptable levels. Untransformed means are reported for ease of interpretation. Descriptive analyses were conducted to summarize subject characteristics. Tests for departure from Hardy–Weinberg equilibrium were performed via chi-square test for goodness of fit [38]. A separate 3 (genotype) × 3 (time) analysis of variance (ANOVA) with repeated measures explored genotype differences across time on each endpoint (cortisol, HR, SBP, and DBP) for each of the three polymorphisms (5HTTLPR, Bcl1, and −2C/G). Genotype groupings were then decomposed into alleles, after which a separate 2 (allele) × 3 (time) ANOVA compared carriers versus non-carriers of target alleles within each polymorphism on each endpoint following either a dominant (e.g., if known risk factor = G, then GX + GG vs. XX) or recessive model (if risk factor = G, then GG vs. GX + XX) [38]. In the absence of clear evidence for allelic dominance in the extant literature, allele groupings were selected based on visual inspection of the 3 (genotype) × 3 (time) plots. In ambiguous cases, both dominant and recessive models were tested. Finally, in cases where more than one polymorphism influenced a given endpoint, "high" and "low" composite genotype groups were computed, followed by a separate 2 (genotype group) × 3 (time) ANOVA to evaluate additive effects (inclusion cut point $p < .100$). Greenhouse–Geisser corrections were implemented when sphericity assumptions were not met. Post hoc independent t tests further explored overall time effects as well as the group effects at each time point for the allele group comparisons. Absolute (value 2 − value 1) and relative Δ scores ([value 2 − value 1/value 1] × 100%) were also computed and then compared across groups as exploratory post hoc independent t tests. These calculations were performed to more specifically explore "reactivity" (i.e., initial response from baseline to mock-captivity stress), "recovery" (i.e., change from mock-captivity stress to 24-h recovery), and "residual elevation," (i.e., sustained disruption from baseline to 24-h recovery). All formal hypothesis tests were two-sided, and the probability of committing a Type I error was set at .05. Effect sizes were estimated via partial eta-squared (η_p^2; [39]), and observed power was computed.

3. Results

3.1. Subject characteristics

Subjects were healthy, male, active-duty Navy and Marine Corps members (39% officers, 60.3% enlisted, and 0.7% missing). Mean ± SEM age, body mass index, and years of military service for this sample were 25.2 ± 0.4 years, 24.9 ± 0.2 kg/m^2, and 4.6 ± 0.3 years, respectively. Most subjects were Caucasian (66.9%). Demographically, this sample is comparable to that of our previously published studies of male survival trainees [31].

For 5HTTLPR, allelic discrimination analysis identified 29 subjects (20.6%) homozygous for the short allele, 50 homozygous for the long allele (35.5%), and 62 heterozygotes (44.0%). For Bcl1, 62 subjects (44.0%) were homozygous for the C allele, 17 (12.0%) were G homozygotes, and 62 (44.0%) were heterozygotes. For −2C/G, 40 subjects (27.8%) were homozygous for the C allele, 46 (32.2%) were G homozygotes, and 57 (40.0%) were heterozygotes. This sample did not depart from Hardy–Weinberg equilibrium with respect to 5HTTLPR (χ^2 (1, $N = 141$) = 1.25, $p = .54$) or Bcl1 (χ^2 (1, $N = 141$) = .06, $p = .97$). Marginal evidence of possible departure was observed for −2G/C (χ^2 (1, $N = 143$) = 5.8, $p = .055$), with a deficit in the number of observed heterozygotes.

3.2. Overall effects of intense stress exposure

Exposure to mock captivity substantially disrupted all four endpoints. Overall time effects were observed for cortisol, ($F(2,272) = 378.2$, $p < .001$, $\eta_p^2 = 0.74$, $1 - \beta = 1.00$), HR ($F(1.5, 210.4) = 609.4$, $p < .001$, $\eta_p^2 = 0.81$, $1 - \beta = 1.00$), SBP ($F(1.7, 228.8) = 308.7$, $p < .001$, $\eta_p^2 = 0.69$, $1 - \beta = 1.00$), and DBP ($F(1.8, 251.8) = 56.1$, $p < .001$, $\eta_p^2 = 0.29$, $1 - \beta = 1.00$). Cortisol, HR, SBP and DBP increased an average of 255.9% (0.45 ng/dL), 66.8% (40.3 beats per minute), 22.1% (25.2 mm Hg), and 13.0% (9.2 mm Hg), respectively, from T1 to T2. Mean residual elevation values (T1 − T3 Δ) for HR, SBP, and DBP were 2.5 beats per minute (5.5%), 0.7 mm Hg (1.0%), and 3.0 mm Hg (4.9%), respectively. By contrast, cortisol rebounded substantially (mean T1 − T3 $\Delta = -0.08$ μg/dL [−22.5%]).

Table 1
Genotypes and allele comparisons: cortisol stress trajectories.

Polymorphism	*N*	Salivary cortisol (μg/dL)			*p*
		Baseline *M* (SEM)	Stress *M* (SEM)	Recovery *M* (SEM)	
5HTTLPR					
Genotype					
SS	27	0.27 (0.02)	0.73 (0.07)	0.18 (0.02)	
LL	48	0.22 (0.01)	0.61 (0.05)	0.15 (0.01)	
SL	60	0.23 (0.02)	0.71 (0.05)	0.15 (0.01)	.041
Allele					
L carrier	108	0.22 (0.01)	0.66 (0.03)	0.15 (0.01)	
L non-carrier	27	0.27 (0.02)	0.73 (0.07)	0.18 (0.02)	.022
Bcl1					
Genotype					
CC	60	0.25 (0.02)	0.70 (0.04)	0.16 (0.01)	
GG	17	0.20 (0.01)	0.62 (0.08)	0.13 (0.01)	
CG	58	0.23 (0.01)	0.67 (0.05)	0.15 (0.01)	.133
Allele					
G carrier	75	0.22 (0.01)	0.66 (0.04)	0.15 (0.01)	
G non-carrier	60	0.25 (0.02)	0.70 (0.04)	0.16 (0.01)	.071
MR −2C/G					
Genotype					
CC	40	0.25 (0.02)	0.74 (0.06)	0.15 (0.01)	
GG	43	0.22 (0.02)	0.55 (0.03)	0.15 (0.01)	
GC	54	0.23 (0.01)	0.73 (0.05)	0.16 (0.01)	.185
Allele					
C carrier	94	0.24 (0.02)	0.73 (0.04)	0.16 (0.01)	
C non-carrier	43	0.22 (0.02)	0.55 (0.03)	0.15 (0.01)	.066

3.3. Genetic variants and acute stress trajectories

Genotype and allele comparisons on adrenocortical and cardiovascular endpoints are detailed in Tables 1–4. As shown in Table 1, overall group effects emerged for the 5HTTLPR genotype on cortisol ($F(2,132) = 3.3$, $p = .041$, $\eta_p^2 = 0.05$, $1 - \beta = 0.61$). Testing a recessive model for the short variant, SS revealed higher overall cortisol concentrations than L carriers ($F(1,133) = 5.4$, $p = .022$, $\eta_p^2 = 0.04$, $1 - \beta = 0.64$), with post hoc paired t tests suggesting marked influence at T1 ($t(138) = 2.4$, $p = .017$). These groups did not differ on absolute or relative reactivity, recovery, or residual elevation. Despite noteworthy trends, no genotype or allele effects on cortisol prevailed for Bcl1 ($p = .071$) or −2C/G ($p = .066$).

As shown in Table 2, a nuanced interaction effect emerged for 5HTTLPR on HR for both the genotype comparison ($F(3.1, 206.7) = 2.8$, $p = .04$, $\eta_p^2 = 0.04$, $1 - \beta = 0.68$) and the recessive model for the short variant (SS versus LS + LL; $F(1.5,207.6) = 4.2$, $p = .026$, $\eta_p^2 = 0.03$, $1 - \beta = 0.65$). Specifically, L carriers demonstrated higher mean stress-induced HR (T2) than SS yet rebounded to a lower mean recovery value (T3). Post hoc t tests identified group differences at T3 ($t(135) = 2.4$, $p = .016$), greater reactivity quantified by larger relative T1 − T2 Δ (+69.6% vs. +56.3%; $t(135) = -2.0$, $p = .044$),

Table 2
Genotypes and allele comparisons: heart rate stress trajectories.

Polymorphism	*N*	Heart rate (beats per minute)			*p*
		Baseline *M* (SEM)	Stress *M* (SEM)	Recovery *M* (SEM)	
5HTTLPR					
Genotype					
SS	28	63.3 (2.1)	99.0 (4.5)	68.6 (2.2)	
LL	48	63.2 (1.4)	102.1 (2.5)	64.6 (1.4)	
SL	61	60.9 (1.3)	104.3 (2.2)	63.0 (1.1)	.040
Allele					
L carrier	109	61.9 (1.0)	103.3 (1.6)	63.7 (0.9)	
L non-carrier	28	63.3 (2.1)	99.0 (4.5)	68.6 (2.2)	.026
Bcl1					
Genotype					
CC	60	62.5 (1.3)	100.1 (2.2)	65.4 (1.1)	
GG	17	65.5 (2.3)	111.2 (5.2)	65.5 (2.8)	
CG	60	60.6 (1.3)	103.0 (2.4)	63.3 (1.3)	.083
Allele					
G carrier	77	61.7 (1.1)	104.8 (2.2)	63.8 (1.2)	
G non-carrier	60	62.5 (1.3)	100.0 (2.2)	65.4 (1.1)	.047
MR −2C/G					
Genotype					
CC	40	64.0 (1.7)	103.2 (3.2)	63.6 (1.5)	
GG	45	61.8 (1.5)	99.3 (2.7)	65.5 (1.5)	
GC	54	61.1 (1.3)	105.3 (2.4)	64.8 (1.3)	.107
Allele					
C carrier	94	62.4 (1.0)	104.4 (1.9)	64.2 (1.0)	
C non-carrier	45	61.8 (1.5)	99.3 (2.7)	65.5 (1.5)	.085

Table 3
Genotype and allele comparisons: SBP stress trajectories.

Polymorphism	*N*	SBP (mm Hg)			*p*
		Baseline *M* (SEM)	Stress *M* (SEM)	Recovery *M* (SEM)	
5HTTLPR					
Genotype					
SS	28	117.7 (1.6)	140.4 (2.8)	119.0 (1.5)	
LL	48	114.6 (1.6)	141.4 (2.3)	114.2 (1.5)	
SL	60	116.6 (1.3)	140.9 (2.2)	117.4 (1.2)	.541
Allele					
L carrier	108	115.7 (1.0)	141.2 (1.6)	116.0 (1.0)	
L non-carrier	28	117.7 (1.6)	140.4 (2.8)	119.0 (1.5)	.481
Bcl1					
Genotype					
CC	60	115.4 (1.3)	141.8 (2.1)	115.9 (1.3)	
GG	17	121.1 (2.9)	147.2 (4.7)	116.9 (2.3)	
CG	59	114.9 (1.3)	138.3 (1.8)	117.3 (1.2)	.100
Allele					
C carrier	119	115.1 (0.9)	140.1 (1.4)	116.6 (0.9)	
C non-carrier	17	121.1 (2.9)	147.2 (4.7)	116.9 (2.3)	.070
MR −2C/G					
Genotype					
CC	40	115.8 (1.8)	140.2 (2.8)	115.8 (1.5)	
GG	43	114.9 (1.5)	140.0 (2.3)	115.0 (1.7)	
GC	55	116.8 (1.3)	142.2 (2.1)	118.3 (1.1)	.393
Allele					
C carrier	95	116.4 (1.1)	141.4 (1.7)	117.2 (0.9)	
C non-carrier	43	114.9 (1.5)	140.0 (2.3)	115.0 (1.7)	.324

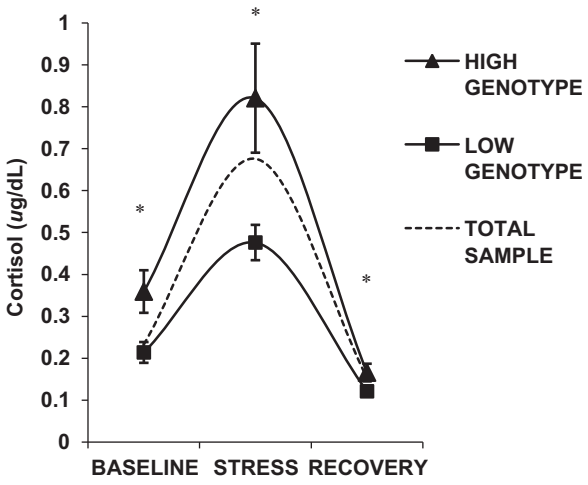
as well as superior recovery quantified by larger absolute (−39.6 beats per minute vs. −30.4 beats per minute; $t(135) = 2.5$, $p = 0.014$) and relative T2 − T3 Δ (−37.0% vs. −27.9%; $t(135) = 3.3$, $p = .001$). For Bcl1, a test of a dominant model for the G allele (GC + GG versus CC) suggested higher mean stress-induced HRs (T2) in G carriers but not at baseline or recovery (interaction effect: $F(1.5, 202.9) = 3.5$, $p = .047$, $\eta_p^2 = 0.025$, $1 - \beta = 0.56$). Post hoc comparisons, in turn, suggested greater reactivity in G carriers quantified by larger absolute T1 − T2 Δ (+43.1 beats per minute vs. +37.6 beats per minute; $t(135) = -1.9$, $p = .050$), as well as greater recovery shown by larger absolute (−40.1 beats per minute vs. −34.7 beats per minute; $t(135) = 2.1$, $p = .042$) and relative T2 − T3 Δ (−37.5% vs. −32.8%; $t(135) = 2.1$, $p = .041$). No effect of −2C/G on HR prevailed.

As seen in Table 3, a test of a recessive model for the G allele suggested higher overall SBP values in homozygous G carriers of Bcl1 compared with C carriers (CG + CC), but this trend did not reach statistical significance ($p = .070$). Neither 5HTTLPR nor −2C/G substantively influenced SBP.

As shown in Table 4, DBP was influenced by all three polymorphisms. Testing a dominant model for the 5HTTLPR S variant, carriers (SS + SL) showed higher overall DBP than non-carriers (LL) (group effect: $F(1,134) = 4.2$, $p = .043$, $\eta_p^2 = 0.030$, $1 - \beta = 0.53$).

Table 4
Genotype and allele comparisons: DBP stress trajectories.

Polymorphism	*N*	DBP (mm Hg)			*p*
		Baseline *M* (SEM)	Stress *M* (SEM)	Recovery *M* (SEM)	
5HTTLPR					
Genotype					
SS	28	77.5 (1.6)	86.4 (2.2)	79.4 (1.4)	
LL	48	74.3 (1.1)	84.1 (1.5)	76.5 (0.9)	
SL	60	76.1 (1.0)	85.3 (1.3)	79.4 (1.0)	.109
Allele					
S carrier	88	76.6 (0.8)	85.6 (1.1)	79.4 (0.8)	
S non-carrier	48	74.3 (1.1)	84.1 (1.5)	76.5 (0.9)	.043
Bcl1					
Genotype					
CC	60	75.2 (1.0)	82.6 (1.2)	77.7 (1.1)	
GG	17	79.4 (2.1)	87.1 (2.5)	80.8 (1.5)	
CG	59	74.4 (1.1)	86.1 (1.4)	78.9 (0.9)	.061
Allele					
C carrier	119	74.8 (0.7)	84.4 (1.0)	78.3 (0.7)	
C non-carrier	17	79.4 (2.1)	87.1 (2.5)	80.8 (1.5)	.039
MR −2C/G					
Genotype					
CC	40	73.9 (1.5)	83.0 (1.6)	76.7 (1.1)	
GG	43	76.4 (1.1)	85.1 (1.7)	79.2 (1.1)	
GC	55	75.9 (0.9)	86.3 (1.4)	79.3 (1.0)	.089
Allele					
G carrier	98	76.1 (0.7)	85.8 (1.1)	79.2 (0.8)	
G non-carrier	40	73.9 (1.5)	83.0 (1.6)	76.7 (1.1)	.028



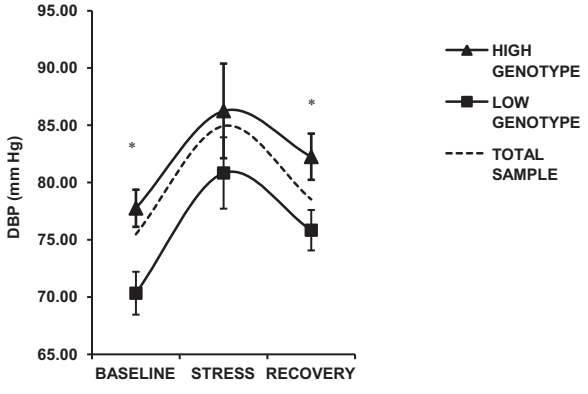
*$p < .05$.

Fig. 1. High versus low genotype groups: cortisol stress trajectory.

Post hoc comparisons identified higher DBP in S carriers at T3 ($t(134) = -2.2$, $p = .029$). For Bcl1, a recessive model for allele G showed higher overall DBP in GG compared with C carriers (CC + GC) (group effect: $F(1,134) = 4.3$, $p = .039$, $\eta_p^2 = 0.031$, $1 - \beta = 0.54$), while post hoc comparison located the group difference at T1 ($t(134) = 2.2$, $p = .026$). For −2C/G, a dominant model for allele G showed higher overall DBP in G carriers (GG + GC) than CC (group effect: $F(1,136) = 4.9$, $p = .028$, $\eta_p^2 = 0.035$, $1 - \beta = 0.60$), while the post hoc comparison located a marginally significant group difference at T3 ($t(136) = -1.9$, $p = .063$).

As noted above, high and low genotype groups were constructed from polymorphisms that plausibly influenced each endpoint (Tables 1–4). High genotype groups were constructed by summing constituent 5HTTLPR, Bcl1, and −2C/G genotypes as follows: cortisol [SS] + [CC] + [CC + CG]; HR [SS] + [GG + GC] + [CC + CG]; and DBP [SS + LS] + [GG] + [GG + GC]. Accordingly, low genotype groups were constructed as follows: cortisol [LL + LS] + [GG + GC] + [GG]; HR [LL + LS] + [CC] + [GG]; and DBP [LL] + [CC + CG] + [CC]. High versus low genotype group comparisons are depicted in Figs. 1 and 2.

As shown in Fig. 1, high ($n = 8$) and low genotype groups ($n = 18$) revealed different cortisol trajectories, with substantially higher cortisol concentrations in the high genotype group at all three time points (group effect: $F(1,24) = 18.4$, $p < .001$, $\eta_p^2 = 0.43$, $1 - \beta = 0.98$). Post hoc t tests reiterated a distinct influence at T1 ($t(26) = -2.7$, $p = .012$), T2 ($t(26) = -3.1$, $p = .004$) and T3 ($t(24) = -2.4$, $p = .025$). No such differences prevailed with respect to absolute or relative reactivity, recovery, or residual elevation. High and low genotype groups did not differ on HR trajectories. Also, genotype groups were not constructed for SBP because only one polymorphism showed plausible relevance to the SBP trajectory (Table 3). As shown in Fig. 2, high ($n = 8$) and low genotype groups ($n = 12$) showed different DBP trajectories, with substantially higher DBP in high genotype groups at all three time points (group effect: $F(1,18) = 5.0$, $p = .037$, $\eta_p^2 = 0.22$, $1 - \beta = 0.57$). Post hoc t tests identified a distinct influence at T1 ($t(18) = -2.8$, $p = .012$) and T3 ($t(18) = -2.4$, $p = .030$). High and low genotype groups did not differ on absolute or relative DBP reactivity, recovery, or residual elevation. Total sample means at each time point are provided in both figures for visual inspection only.



*$p < .05$.

Fig. 2. High versus low genotype groups: DBP stress trajectory.

4. Discussion

The purpose of this study was to evaluate unique and combined effects of polymorphisms in the serotonin transporter, glucocorticoid receptor, and mineralocorticoid receptor genes on adrenocortical and cardiovascular stress responses. The cortisol trajectory was modulated most convincingly by 5HTTLPR; HR was influenced by 5HTTLPR and Bcl1; and DBP was affected by all three polymorphisms. Remarkable differences between high and low genotype groups on cortisol and DBP trajectories suggested a synergistic effect of these polymorphisms. Pending additional study, these findings may inform drug discovery, gene therapy, and stress inoculation strategies.

As hypothesized, 5HTTLPR SS subjects demonstrated greater overall cortisol concentrations than L carriers. This is partially consistent with an accruing literature linking the S allele to increased cortisol reactivity to laboratory stressors [5,7,8]. However, although the present study showed overall group differences, no differences were identified with respect to reactivity, recovery, or residual elevation. Since these three characteristics have been implicated in clinical models of stress and disease [1], future research in ecologically valid settings is needed to clarify whether or not heightened cortisol concentrations across the stress trajectory in S-carriers are a function of baseline values. This may elucidate the clinical significance of the 5HTTLPR-cortisol relationship.

DBP was convincingly modulated by all three polymorphisms. Specifically, 5HTTLPR S carriers showed higher overall values than non-carriers, Bcl1 GG were higher than C carriers, and −2C/G G carriers exceeded non-carriers. This fuels current disagreement in the literature regarding the role of 5HTTLPR and BP reactivity. Whereas Ohira et al. [10] have shown stronger SBP and DBP responses to mental stress in SS compared with SL and LL in men, Williams and colleagues have linked the L allele to increased SBP, DBP [11], and mean arterial pressure [12] reactivity to mental stress in healthy volunteers. Plausible sources of discrepancy across these studies include different ethnic and demographic characteristics of subjects as well as contextual differences across studies.

Although additive effects of the candidate polymorphisms were anticipated, remarkable differences between high and low genotype groups on cortisol and DBP trajectories implied a synergistic effect. This is the most compelling pair of findings of the current study, because striking increases in effect sizes were observed when individual genotypes were combined into high and low genotype groups. In the case of cortisol, considerable enhancement in statistical power was achieved in the genotype group model despite dramatic decreases in sample size. This supports and extends the foundational argument that many different genetic variants converge to influence a given stress profile, with each individual variant making a subtle yet stable contribution [40].

Limitations of this study should be recognized. A relatively modest sample size was studied; as a result, observed power for statistically significant genotype and allelic comparisons ranged from 0.53 to 0.68. Power of 0.70–0.80 (typically preferred) would likely have revealed more robust statistical significance for each primary analysis. In future studies, larger sample sizes and enhanced statistical power will permit more sophisticated analysis of genotype interactions (thus more rigorously quantifying synergistic effects), and will also support quantitative analyses of gene–dose effects (i.e., if risk factor = G, then GG > GC > CC) [38]. Despite these limitations, this study demonstrates compelling evidence for genetic modulation of the human stress response in an ecologically valid context.

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14. ABSTRACT

Context Common variants in serotonin and corticosteroid receptor genes influence human stress in laboratory settings. Little is known of their combined effects.
Objective This study evaluated distinct and combined effects of polymorphisms in the serotonin transporter (5HTTLPR L/S), glucocorticoid receptor (Bcl1 C/G), and mineralocorticoid (-2C/G) receptor genes on adrenocortical and cardiovascular reactions to intense, realistic stress.
Setting The study took place within a 12-day military survival course.
Design Participants were studied before, during, and 24 hours after the course.
Participants One hundred forty-four healthy, active-duty military men were studied.
Main Outcome Measures Dependent variables were cortisol, heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP).
Results 5HTTLPR SS carriers revealed higher overall cortisol concentrations than L-carriers (p = .022). 5HTTLPR L-carriers demonstrated higher stress-induced HR than non-carriers (SS) yet rebounded to a lower recovery value (p = .026), while Bcl1 G carriers showed higher mean stress-induced HR than non-carriers (CC) (p = .047). For DBP, 5HTTLPR S carriers showed higher overall values than non-carriers (LL) (p = .043), Bcl1 GG were higher than C carriers (p = .039), and -2C/G G carriers exceeded non-carriers (CC) (p = .028). A "high" haplotype revealed substantially higher overall cortisol concentrations than a "low" haplotype (p < .001), as was the case for DBP (p = .037).
Conclusion: This study revealed synergistic effects of common polymorphisms on human responses to intense stress. These findings may have implications for drug discovery, gene therapy, and stress inoculation strategies.

15. SUBJECT TERMS
stress, military, survival, genotypes, polymorphisms, cortisol, blood pressure

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